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REACTIONS AND RESISTANCE OF FISHES IN THEIR NATURAL ENVIRONMENT TO ACIDITY, ALKALINITY AND NEUTRALITY.

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I. INTRODUCTION.

The present paper is the first of a series that is to deal with the relation of fishes to ions in the natural environments. It is purposed to point out some of the close correlations which exist between the physiology of fishes and their behavior, and to present evidence concerning the importance of such correlations in biological investigation in general. The data presented in the following pages deal with the reactions and resistance of fresh water fishes to acidity, neutrality and alkalinity;

the discussion of the data shows that the phenomena outlined receive much support from the work of other investigators and that the environmental factors which are important to fresh water fishes are probably of importance to many, if not all, other organisms as well.

The investigation has been carried on at the University of Illinois, in Professor V. E. Shelford's laboratory. The work has been done in connection with another line of inquiry regarding the reactions and resistance of fishes to salts. The results of this second investigation will appear as the second paper of the series.

II. APPARATUS AND METHODS.

Two general types of experiments have been run, namely, reaction experiments, and resistance experiments.

A. Reaction Experiments.

This method of experimentation was devised by Shelford and Allee ('13) and may be designated as the "gradient method." In brief the procedure is as follows: A solution gradient is established in an observation tank, the fish introduced, and its movements graphed. The graph, together with notes taken at the time, makes up the experimental record. Fig. 1 shows the type of tank used. A similar tank was used by Shelford and Powers ('15) in their experiments with marine fishes. A black hood screens the tank, the movements of the fishes being viewed through slits in the front of the hood.

The tank has a plate glass front and is lighted by symmetrical lights placed above. A plate-glass cover fits into the top and rests against the surface of the water. This cover is useful in experiments with gaseous gradients as it lessens the vertical gradient due to escape of gas at the surface.

The water flows into the tank through the openings (inlets) in the ends, then toward the middle; at the middle the water from the two ends mixes, the water from each end drifting somewhat past the middle, thus forming the gradient. The water flows out through the exits (outlets) in the bottom and at the top of the tank. An experiment consists of first establishing the gradient, and then introducing the fish and graphing its movements.

In establishing the gradient the flow at each end of the tank was fixed at 500 c.c. per minute in practically all the experiments. The flow of tap water was regulated to 500 c.c. per min. at one

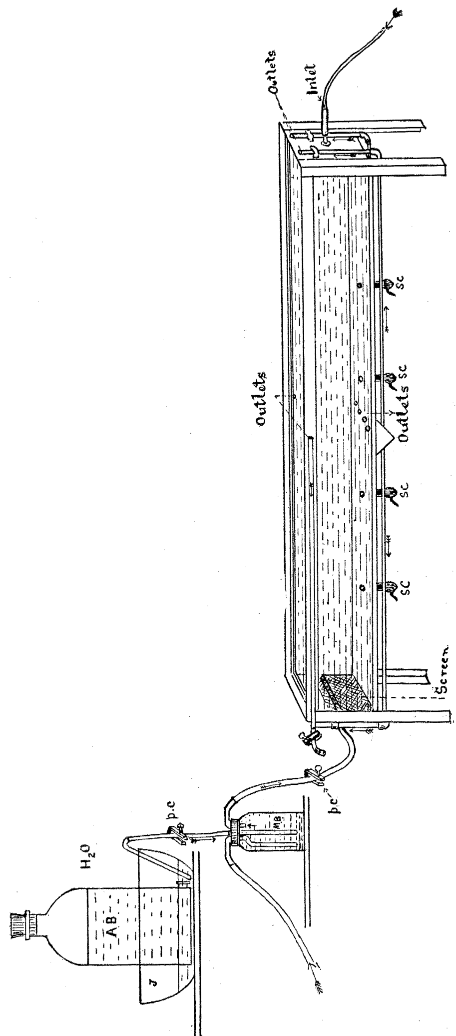

FIG. 1. Showing the gradient tank in which the reaction experiments were performed. The water flowed in at the *inlet* in each end, was distributed by the brass tee (T), drifted slowly to the middle, diffusing across somewhat, and out through the *outlets*. One fourth flowed out through each of the two outlets at the top and one eighth through each of the four at the bottom. The experimental factor was introduced from the aspirator bottle (AB). This bottle was filled with a solution five times as concentrated as that desired in the experiment. The solution was siphoned out of the jar (J) at the rate of 100 c.c. per min. into a mixing bottle (MB) into which tap water was flowing at the rate of 400 c.c. per min. This gave a flow into the tank, of 500 c.c. per min., and the desired concentration. Tap water, or water modified to a different degree, flowed into the opposite end at the same rate. Samples for testing were withdrawn through the stopcocks (SC) or from above by means of a pipette.

end and to 400 c.c. per min. at the other. Then 100 c.c. per min. of a solution of treated water was introduced into the 400 c.c. flow from a mixing bottle ($M B$). The 100 c.c. flow was kept

constant by using an aspirator bottle as in Fig. 1 (*A B*). This bottle was filled with treated water, corked at the top and placed in the jar (*J*). The water was siphoned from *J* and the pressure was constant as the solution escaped from *A B*, when the level began to fall in *J*. The strength of the solution in the aspirator bottle was always five times that desired in the treated water end of the tank.

The following variations of the simple graphing method of recording were used by Shelford and Allee and have been introduced here. (1) In experiments where the fish was decidedly inactive and remained in one end of the tank, it was driven into the opposite end with a rubber-tipped glass rod. The driving was done at regular intervals and was repeated at similar intervals in the controls. Dotted lines in the graphs indicate that the fish was driven. Experiments of this sort were few in number and have for the most part been thrown out. In some experiments, however, the fishes were active and yet remained constantly in one end of the tank. Driving was again resorted to, in some cases, to make sure that the selection of the given end was a reaction to the gradient. A return to the original end would indicate this to be the case. (2) A number of experiments was performed with 4–10 small fishes in the tank at the same time. These experiments were recorded by readings taken 30 seconds apart. The readings indicate per cent. of fishes in each third of the tank at the time of reading. (3) Usually the fishes were not placed in the tank until the flow at the ends had been going for some time. Thus a gradient was established before the fishes were introduced. In some cases, however, the fishes were either left in the tank when the ends were reversed, or introduced before a gradient had formed. The results of these experiments do not differ from the others except in per cent. of time spent in the thirds of the tank.

The controls were blank experiments, run with untreated water flowing in at both ends, or with no flow at either end. Experiments with the treated water first at one end and then at the other, also served as controls.

The gradient was determined by simple titration with standard acid or alkali, using phenolphthalein or methyl orange

(or both) as indicator. The samples were collected by means of a pipette inserted to a given level below the surface, and the titrations were always made at once, with as much care as seemed necessary. For instance samples containing a high concentration of CO_2 need to be titrated with rapidity, while samples containing H_2SO_4 or KOH may be titrated without haste.

B. Resistance Experiments.

The procedure in the resistance experiments was very simple in most cases. In general the desired solutions were made up from standard solutions of the acid or alkali (measured from burettes) and the fishes introduced. Temperature was controlled by setting the jars containing the solutions, in running tap water. As the experiment proceeded, samples for testing were withdrawn when necessary, and the same amount of water was removed from the control. General controls were kept running throughout the entire time, while numerous temporary controls were set up as demanded by individual experiments.

The species of fishes used principally, have been the blue-gill (*Lepomis pallidus*), white crappie (*Pomoxis annularis*), green spotted sun-fish (*Lepomis cyanellus*), and bull-head (*Ameiurus melas*). Most of the fishes were caught (by seining) in the small streams in the vicinity of the university (the crappie came from a small artificial lake); all were brought into the laboratory with as little handling as possible, and placed at once in large aquaria. They were fed from day to day but fishes do not always eat well in confinement and as time went by they became more or less starved. The changes in the reactions of fishes, which accompany starvation have been investigated and will be discussed in another paper.

The chemicals used have been the chemically pure preparations of Kahlbaum or the analyzed preparations of Baker.

III. The Water.

An investigation of the reactions of fishes to salts in solution was begun at Chicago in 1912, at the suggestion of Dr. Shelford. In the fall of 1914 Dr. Shelford left Chicago to take a position at the University of Illinois and the writer accompanied him to

continue the work in his laboratory at that place. The differences in the water supply of the two institutions brought up a number of new questions regarding the reactions of the fishes, and it was decided that to continue the investigation satisfactorily, more must be known of the effects of acid and alkaline water upon fishes and their reactions. This second investigation was therefore taken up and the results are published in advance of those with the salts, since they bear directly upon the interpretation of the latter. A brief comparison of the water of the two institutions will be profitable at this point.

The water at Chicago is pumped from Lake Michigan and analyses show it to be considerably different in gaseous and solid content from the water at Illinois, which comes from deep wells. In 1912 Allee, who had been working at Chicago on rheotaxis in isopods, came to Illinois, bringing with him a stock of animals. In his paper ('13) he compares the waters of the two institutions, and gives a table showing the differences in the dissolved content. This table is inserted here.

TABLE I.

A COMPARISON OF CHEMICAL ANALYSES OF CHICAGO AND UNIVERSITY OF ILLINOIS TAP WATER.

Analysis of solids in parts per million, and gases in cubic centimeters per liter.

	Chicago Tap.	U. of I. Tap.
Potassium, K	6.0	2.6
Sodium, Na	42.1	29.0
Ammonium, NH_4	0.04	2.3
Magnesium, Mg	11.3	34.9
Calcium, Ca	34.6	70.1
Iron, Fe	0.15	1.0
Aluminum, Al	0.00	1.3
Silica, Si	3.3	18.9
Nitrate, NO_3	1.7	0.7
Chlorine, Cl	12.0	3.5
Lead, Pb	0.01	0.00
Sulphuric acid, SO_3	0.04	2.3
Oxygen, O	10.46	0.12
Free carbon dioxide, CO_2	2.5	18.0
Half bound CO_2 (bicarbonates)	32.5	101.12

Allee states that the change of water did not greatly affect the rheotactic response of the isopods. At Illinois he kept the

animals in aërated water, which was thus saturated with oxygen (5.5 to 7 c.c. per liter) while the free carbon dioxide was removed. If aërated sufficiently, the Illinois tap water becomes alkaline to phenolphthalein, and upon writing to Allee with regard to this matter he gives me permission to state that he kept his stock of isopods in such alkaline water for a period of 22 weeks, without increased mortality. He points out that the per cent. of rheotactic response, after a large number of trials, was 8 per cent. less than at Chicago but does not know whether or not to regard this as significant.

Table I. shows that Illinois water contains 18 c.c. of CO_2 per liter and practically no oxygen. Either of these conditions would alone prove fatal to fishes, while the combination would be doubly fatal (Wells, '13). Since aëration removes the CO_2 and at the same time saturates with oxygen, it was thought that this would fit the water for supplying the fish aquaria. The water as it came from the tap was therefore run through the aërating pans which form a part of the apparatus described by Shelford and Allee ('13). The device consists of a series of galvanized pans, placed one beneath the other. The water runs into the upper pan and trickles down through successive pans into a galvanized tank. From the tank, pipes lead to the aquaria. The flow into two large aquaria was regulated to 500 c.c. per minute for each and the fishes were now brought in from the nearby streams and placed in the aquaria in rather large numbers. The aquaria were 8 ft. x 2 ft.; about 300 small fishes were placed in each. This was overcrowding, but fishes have been kept successfully for some time, in closer quarters at Chicago.

The immediate mortality of the stock was not great. It was noted that the darters and other more sensitive fishes did not live well but the sunfishes, bullheads and minnows seemed to be normal. In a few days, however, these fishes began to die. It was thought that the water contained too large an amount of carbonates and an arrangement was made to introduce sulfuric acid into the aërated water at the galvanized supply tank. Enough acid was added to convert about one third of the carbonates into sulfates and with some benefit. It had been noted in the experiments that the fishes did not swim about as actively as

usual and that their sensitivity seemed to be lessened, as they would swim into factors to which they are normally very negative. It was at this point that the study of the effects of acidity, etc., was decided upon.

Tests showed that the water entering the aquaria was practically neutral to phenolphthalein, varying a little from day to day. To determine the effect of the neutral water upon the fishes, a number was taken from the aquaria and placed in tubs of partially aërated tap water (water contained 6–10 c.c. CO_2 per l.). After a day or so in this water, they began to behave normally in the gradient again. The flow of water into the aquaria was now modified by diminishing the amount of aëration. The tap water was run down a wooden trough 12 ft. long, into the aquaria. This saturated it with oxygen but left it decidedly acid with CO_2. From now on the mortality of the stock of fishes was very low. The aquaria were not so crowded as at first, but that the decrease in the number of fishes does not explain the low mortality, will be brought out in experiments to be presented later.

The importance of the chemical reaction of the water to fishes had been foreseen (Wells, '13, p. 337) and it was decided that the peculiar properties of the Illinois water offered an excellent opportunity for continuing this investigation. At the same time it was thought that the work might perhaps throw some light upon a number of the reactions of fishes to salts, which seemed difficult to explain.

The advantages of the Illinois water are due to the following chemical properties: As it flows from the tap it is acid to phenolphthalein from the excess (18 c.c. per liter) of CO_2, and alkaline to methyl orange because it contains a large quantity (101 c.c. per liter) of bicarbonates in solution. The bicarbonates have been formed from carbonates according to the equation $CaCO_3 + H_2CO_3 \leftrightarrows Ca(HCO_3)_2$ and when carbonates are dissolved under the influence of excess of carbonic acid they are practically all converted into bicarbonate, the quantity of unconverted carbonate being negligible (Stieglitz, '09, p. 246, Seyler, '94, p. 105). Under the pressure in the water pipes, there exists an equilibrium between the carbonic acid and the bicarbonates,

but when the water flows out of the tap, the pressure is removed and the carbonic acid at once begins to dissociate into CO_2 and water. The CO_2 passes off into the air and the dissociation of the acid continues until equilibrium with the CO_2 in the atmosphere is established. Parallel with the dissociation of the carbonic acid there goes an increasing tendency for the bicarbonates to break up to form the normal carbonate, and by the time the acidity from the carbonic acid has diminished to approximate neutrality, the bicarbonates are producing a sufficient quantity of the normal carbonate to give the water an alkaline reaction to phenolphthalein. Thus by regulating the amount of aëration, the water can be left acid, made neutral or even alkaline.

Biologists speak of the carbonates, bicarbonates, and carbonic acid, as fixed, half bound and free CO_2, respectively. The fixed is that existing as simple carbonates, the half bound that necessary to convert the carbonates into bicarbonates, and the free that remaining in excess (Seyler, '94, p. 104). It will be seen that the bicarbonates contain both fixed and half bound CO_2, *i. e.*, CO_2 which is to become half bound is added to CO_2, that is already fixed, to form the bicarbonates. Failure to recognize this fact often leads to confusion when these terms are used.

The amounts of the three kinds of CO_2 can be determined accurately by titration, using two indicators, phenolphthalein and methyl orange. Methyl orange is unaffected by H_2CO_3 and hence the bases present as carbonates or bicarbonates can at once be titrated with acid. Carbonates are alkaline to phenolphthalein, bicarbonates are neutral, and free CO_2 is acid. A carbonate titrated with acid, therefore, becomes neutral to phenolphthalein (if titrated under conditions which prevent loss of CO_2) when the carbonates have all been converted into bicarbonates.

Methyl orange is not affected by H_2CO_3 because this acid does not produce a high enough concentration of H ion. The indicator is however very sensitive to OH ion and reacts to the minute amounts that are present in a bicarbonate solution. Phenolphthalein, on the other hand, is very sensitive to H ion

but not to OH ion. It therefore gives an acid reaction with CO_2 but is unaffected by the minute amount of OH ion which is present in solutions of bicarbonates.[1] Methyl orange will give an alkaline reaction in water in which the concentration of H ion is considerably greater than that of OH ion. Thus, in the presence of CO_2, titration with this indicator is not a determination of true alkalinity for the water is as a matter of fact acid, since it contains a higher concentration of H than OH ions. Marsh ('07) makes this error when he states (p. 337) that "the reaction of water which will support fish life must be slightly alkaline." His determinations were made with sulfuric acid, using methyl orange as indicator. The water to which he refers ("Potomac service water") was in all probability acid to phenolphthalein. Marsh also states that "when the water becomes even slightly acid, fishes cannot live in it." This would mean that fishes can not live in water which has been made slightly acid to methyl orange by the addition of an acid. I have added sulfuric acid to tap water until it gave an acid reaction to methyl orange, and find that fishes live in it as well as in the original tap water, *i. e.*, normally. The fishes should not be placed in such water until some little time after the adding of the sulfuric acid, however, for in the process of changing the carbonates to sulfates, a large amount of carbonic acid is liberated ($CaCO_3 + H_2SO_4 \leftrightarrows CaSO_4 + H_2CO_3$) and until this carbonic acid has dissociated and the CO_2 passed off into the atmosphere to a large degree, its presence will kill fishes which may be introduced. The amount of carbonic acid formed will depend upon the amount of carbonates in the water. (The reaction with bicarbonates will give the same result.)

In the following pages I shall introduce experimental data to show that fresh water fishes cannot live normally in water that is alkaline but that they require a certain degree of acidity to carry on their normal activities.

[1] Noyes ('13) gives a table (p. 388) showing the acidity or alkalinity of solutions at the change of color for various indicators. Methyl orange gives an alkaline reaction when the OH concentration is only 10^{-9} while phenolphthalein is not affected until the concentration of OH ion reaches 10^{-5}. Methyl orange gives an acid reaction when the H ion concentration is 10^{-4} while phenolphthalein reacts when the H ion concentration is only a little more than 10^{-8}.

IV. PRESENTATION OF DATA.

The following experiments show the effect of different degrees of acidity and alkalinity, upon the reactions and longevity of fresh water fishes.

A. Reactions of Fishes to Acidity and Alkalinity.

1. *Reaction to Acids.*

(*a*) *To Carbonic Acid.*—A number of experiments was run to determine the reactions of the fishes to this acid. Three degrees of acidity were used for the most part: (1) Neutral, to very faintly acid (aërated water) (2) moderately acid (8–10 c.c. CO_2 per liter; obtained by using half and half mixture of 1 and 3); (3) strongly acid water (unaërated tap; 18 c.c. CO_2 per liter).

(1) *Moderately Acid Water vs. Strongly Acid Water* (*Graph 1, Chart I.*).—The fishes selected the lower acidity with much precision. They also spent much time at the surface, as is characteristic when the concentration of CO_2 is high.

(2) *Slightly Acid vs. Moderately Acid Water* (*Graph 2, Chart I.*).—The fishes were left in the tank, and the flow altered so that the moderately acid water ran into the end that had previously been strongly acid and neutral water was run into the opposite end. The fishes were graphed after five minutes. They definitely selected the end of the tank into which the neutral water was flowing. Test showed this end to contain 3 c.c. CO_2 per liter. Seven experiments with this combination were run and all gave similar results. Variations were due to specific and size differences. The larger fishes and the crappies and green spotted sunfishes selected a somewhat higher acidity than the smaller fishes, especially the blue-gills.

(3) *6 c.c. CO_2 per Liter vs. 4 c.c. per Liter* (*Graph 3, Chart I*).—The concentrations of CO_2 were obtained by regulating the amounts of aërated and unaërated water. Six experiments were run. The bullheads and blue-gills selected the lower concentration with precision, while the sunfishes and crappies chose the higher end with as much definiteness. Thus, as was seen in (2), the species differ in the optimum CO_2 concentration which they select at this time of year.[1] The difference in

[1] Because of the fact that the resistance of fishes varies with the season (Wells, '14), it is very probable that the CO_2 concentration selected by a given species will show seasonal variations also. This point is yet to be investigated.

specific reaction may be in part a matter of size, as the crappies and sunfishes averaged larger than the blue-gills. Small sunfishes were, however, found to be less sensitive to CO_2 than were blue-gills of the same size. In this case the reaction is correlated with resistance as the sunfishes are more resistant than the blue-gills. The bullheads, however, are perhaps the most resistant of our fresh water fishes yet they are very sensitive to CO_2. The sensitiveness of the bullheads is probably related to the peculiarity of their integument which (Herrick, '02) has chemical perceptors "taste buds" scattered over its entire surface.

So far the fishes had for the most part selected the end of the tank containing the largest proportion of neutral water, *i. e.*, the lowest acidity. To ascertain definitely the reactions to the neutral water, the following experiments were performed.

(4) *Slightly Acid (3 c.c. CO_2 per Liter) vs. Neutral Water.*)—The slightly acid water was obtained by partially aërating the water which flowed into one end. This was done by running it through a galvanized tank which was a part of another piece of apparatus. The gradient tested practical neutrality at one end, and slight acidity at the other. The fishes definitely selected the end containing the CO_2 and were thus negative to the neutral water.

(*b*) *Reactions to Sulfuric Acid.*—It should be pointed out that experiments where other acids than H_2CO_3 are added to water, which contains bicarbonates, are open to misinterpretation if an attempt is made to compare the reactions of the animals to the acids, in this way. The addition of a strong mineral acid to such water does not result in the presence of a hydrogen ion concentration from the mineral acid itself, until all the bicarbonates have been decomposed (*i. e.*, changed to sulfates, etc.) or, in other words, not until the water has become acid to methyl orange. The reaction is a double decomposition, $H_2SO_4 + Ca(HCO_3)_2 \leftrightarrows CaSO_4 + 2H_2CO_3$. Until this reaction is completed, the chief result of adding the mineral acid will be to increase temporarily the concentration of carbonic acid by liberating the fixed and half bound CO_2. The concentration of H ion from this weakly ionized acid will be but a small per cent. of that which would have been obtained from the min-

eral acid itself; yet this will be the only hydrogen ion supply until the bicarbonates have all been changed to sulfate (if sulfuric acid is the mineral acid used). The tap water at Illinois requires approximately 90 c.c. of .1 *N* H_2SO_4 to neutralize the bicarbonates in one liter of water while the Chicago water requires about a third as much. According to the above equation one molecule of the acid liberates 2 molecules of CO_2. Therefore 90 c.c. of .1 *N* acid will liberate 210 c.c. of CO_2. Powers ('13) did not take this reaction into account and speaks of comparing the reactions of crayfishes in gradients of HCl and CO_2. As a matter of fact the amounts of HCl which he added to the Chicago tap water were probably used up in neutralizing the bicarbonates. Thus all his gradients were with carbonic acid, and the differences which he gets are due to the liberation of this acid in excess, in the reaction of the HCl and the bicarbonates.

(1) *H_2SO_4 to Neutralize all Bicarbonates vs. Neutral Water.*— The CO_2 liberated in this gradient made the water so acid that the fishes were soon overcome, and died if not removed from the tank. However at first they gave a decidedly negative reaction to the acid end.

(2) *H_2SO_4 to Liberate 40 c.c. CO_2 per Liter vs. Neutral Water.*— The fishes reacted much as they did in gradients of aërated vs. unaërated water. They were very negative to the acid end.

(3) *H_2SO_4 to Liberate 4 c.c. CO_2 per Liter vs. Neutral Water (Graphs 4 and 5, Chart I.).*—Eighteen experiments of this sort were run. Of the 18 graphs, 14 show that the fishes spent 90 per cent. of the time in the acid half of the tank; 3 show more than 50 per cent. of the time in this half and 1 (small blue-gill) shows an 80 per cent. preference for the neutral end. That the fishes are negative to neutral water is thus confirmed. To ascertain the chemical reaction of the water at the point in the tank where the fishes turned back from the neutral end, numerous samples were titrated from this region, during the experiment. They showed that the water at this point contained about 1 c.c. of CO_2 per liter. The graphs shown in Chart I. are typical for all.

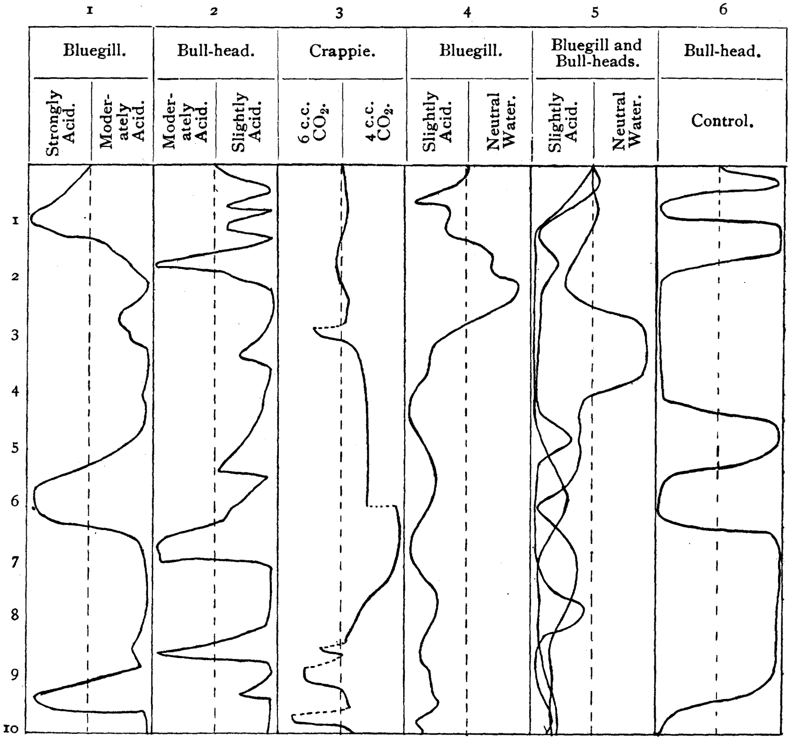

CHART I.

Showing the reactions of the fishes to different degrees of acidity. The gradient is between the two kinds of water, indicated at the top of each graph. Numbers at the left of the chart indicate time in minutes. Strongly acid = 18 c.c. CO_2 per liter; moderately acid = 8–10 c.c. per liter; slightly acid = 2–3 c.c. per liter; and neutral water = actual neutrality to 1 c.c. per liter. Dotted lines indicate that fish was driven.

(*c*) *Reaction to Acidity in Distilled Water.*—The distilled water, which was available in quantity from the chemistry department, was not rapidly toxic to the fishes and since the foregoing results are of some general biological importance, it was decided to repeat the experiments in distilled water. This water was faintly acid with CO_2 containing 2–3 c.c. per liter. It contained no salts; so the addition of a strong acid resulted in no complications such as those discussed in the case of the tap water. A number of experiments was performed with various strengths of acid and alkali. The neutral portion of the tank was kept track of by means of titrations and the reactions of the fishes to this neutral region es-

pecially noted. The results are presented in Table II.; in brief they are as follows. The fishes spent practically all the time in the acid portion of the tank, turning back from the alkaline end at a point just on the acid side of neutrality. They did not, however, select the highest acidity available, but swam back and forth in the tank between neutrality on the one hand and about .0002N H_2SO_4 on the other. The small amount of CO_2 present in the distilled water may be neglected in the presence of the much more ionized acid. At the range of dilution used in these experiments, carbonic acid would have to be about 1,400 times as concentrated as sulfuric acid, to give an equal concentration of H ion.

TABLE II.

SHOWING THE REACTIONS OF FISHES TO ACIDITY AND ALKALINITY IN DISTILLED WATER.

Acid Used.	Concentration.	Reaction.
H_2CO_3	.00004 N	Negative: choose higher acidity.
H_2CO_3	.0001 N	Positive: some fishes choose this concentration in preference to either higher or lower acidity.
H_2SO_4	.0005 N	Very negative.
H_2SO_4	.0002 N	Still very negative.
H_2SO_4	.00005 N	Positive when neutral water is the other choice.

The fishes used did not select alkaline water in any case except when the only other choice was neutrality. Then they spent most of the time on the alkaline side, rather than at the neutral point.

2. *Reactions to Alkalies.*

(*a*) *Alkalies in Neutral Water.* (1) *Na_2CO_3* (.01 N) *in Neutral Water vs. Neutral Water.*—Six experiments were run with this combination. The results were rather indefinite. However, the graphs as a whole show a slight preference for the alkaline half of the tank. As has been pointed out already, the fishes are negative to the neutral water, and these experiments confirm this reaction, even though the only other choice is alkalinity.

(*b*) *Alkalies in Strongly Acid Water.*—In this water which is acid with CO_2 (18 c.c. per liter), the first action of the alkali will be to neutralize the acid. Thus a small amount of alkali introduced at one end will simply produce an acid gradient by

lessening the acidity at this end. Eighteen c.c. of CO_2 equals an .0008 *N* solution. In most cases, the concentrations of alkali used have been much greater than this and the amount used up in neutralizing the acid may be looked upon as negligible. In some experiments, to be cited, the acid factor is of much importance.

(1) *Na_2CO_3 (.01 N) in Strongly Acid Water vs. Strongly Acid Water (Graph* 1, *Chart II.).*—The fishes stayed in the middle of the tank, coming to the surface very little. The gradient was acid at one end and alkaline at the other. Titrations showed that the fishes spent most of the time on the acid side of neutrality.

(2) *Na_2CO_3 (.002 N) in Strongly Acid Water vs. Strongly Acid Water (Graph 2, Chart II.).* Fifteen experiments were run with this combination. The graphs show that the fishes spent most of the time nearer the alkaline end than before, but titration showed that they were merely following the neutral point, remaining on the acid side most of the time.

(3) *Na_2CO_3 (.0005N) in Strongly Acid Water vs. Strongly Acid Water (Graph 3, Chart II.).* This concentration of alkali was just a little more than enough to neutralize the acid in the water of the alkaline end. The end was really slightly acid, however, from the diffusion of more acid from the acid end of the gradient. The fishes moved into the so-called alkaline (really slightly acid) end and remained there during the experiment. This was true for all the fishes used.

(4) *$NaHCO_3$ (.01N) in Strongly Acid Water vs. Strongly Acid Water.*—This salt is neutral to phenolphthalein as has been pointed out in the preceding discussion. A number of experiments, recorded both by graphs and readings at short intervals, were run with it. The results were not at all definite. The fishes seemed to be indifferent to this bicarbonate in acid water, or else they were not at all stimulated by its presence.

(5) *NH_4OH in Moderately Acid Water (Made it Faintly Alkaline) vs. Moderately Acid Water (Graphs 4 and 5, Chart II.).* Ten experiments with this alkali were run, to check up Shelford and Allee's work ('13) with it. They say (p. 252) that the fishes (*Abramis*) did not react to ammonia in a concentration which caused them to turn on their sides after an hour or more. In

my experiments, I found also that the fishes do not react to this alkali with the precision found for the other alkalies used.

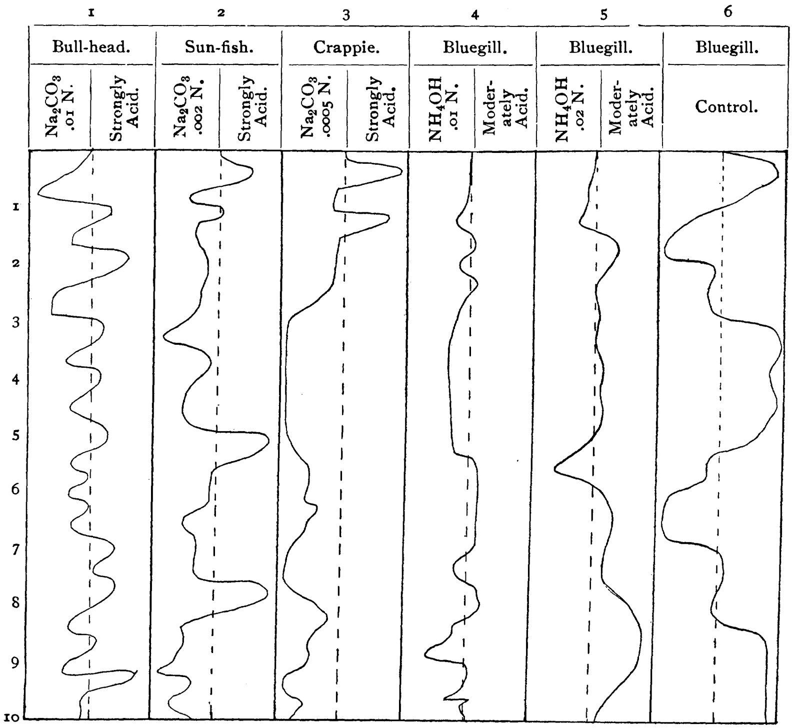

CHART II.

Showing the reactions of the fishes to alkalies. The gradient was between the two kinds of water indicated at the top of each graph. Strongly acid water = 18 c.c. CO_2 per liter; and moderately acid = 8–10 c.c. per liter. Numbers to left = time in minutes.

In the first experiments a .005 *N* solution was run into one end of the tank. The fishes selected the middle of the tank for the most part, though one blue-gill was positive to the ammonia end. The concentration of OH ion was of course very low, with so small a concentration of so weakly ionized a base, and since other experiments have shown that blue-gills are less negative to neutrality than are other fishes, this reaction is not surprising. The ammonia concentration was raised to .01 *N* and the fishes, blue-gill included, moved toward the tap-water end of the tank. Later the concentration of the alkali was

raised to .02 *N*, but even now the avoidance of the alkali end was not nearly so definite as in the experiments with the other alkalies. Graphs 4 and 5 (Chart II.) show this indefinite reaction very clearly. In the .02 *N* gradient, the fishes were soon overcome by the toxicity of the water, which they selected, and they died there if not removed.

The fact that fishes fail to recognize ammonia in solution is of considerable importance, for this substance is being introduced into fish waters in many kinds of sewage. Furthermore it will be shown in the second paper of this series, that the gas has not lost its toxicity even when it has been converted into its various salts. The chemical explanation of the failure of the fishes to recognize and react to the presence of fatal concentrations of the hydrate in solution is probably due to the fact that the concentration of ammonia as gas, reaches a fatal concentration before the concentration of OH ion stimulates the fishes sufficiently to cause them to react negatively. They do not appear to react to the gas itself. Noyes ('13, pp. 203–4) states that ammonia dissolves in water, in part, without chemical change and that it is probable that a large part of the ammonia exists, as such, in the solution. He quotes Moore ('07) as calculating that only 30–40 per cent. of the ammonia exists as ammonium hydroxide, NH_4OH, at 20° C. Noyes thinks that the per cent. may be even less than this.

Again, the solution of ammonia diffuses through the water with great rapidity; much more rapidly than do most other substances. To determine the rate of diffusion, a little phenolphthalein was added to the aspirator bottle (*AB*, Fig. 1) containing the ammonia solution. The pink solution could be seen as it moved through the tank, and in less than a minute it had spread over the entire surface, and to a lesser extent, had penetrated the deeper water. Because of this rapid diffusion, no perfect gradient could be established with this substance. It may also be noted that ammonia behaves just opposite from the salts, the latter spreading along the bottom. In the ammonia experiments, the fishes seldom approach the surface, while in strong carbon dioxide gradients, they spend much time gulping the surface film. Shelford and Allee ('13, p. 231) state

that in open tanks the amount of CO_2 at the surface is markedly less than at deeper levels.

3. *Conclusions Drawn from the Reaction Experiments.*

The reaction experiments recorded in the previous pages suggest the following conclusions. (1) Fresh-water fishes are negative to neutrality in favor of either slight acidity or slight alkalinity. Their normal choice is slight acidity (about .00005 *N* H_2SO_4 or .0001 *N* CO_2). (2) Species of fishes differ in the degree of acidity selected. Blue-gills select water that is but very slightly acid (1–2 c.c. CO_2 per liter, *i. e.*, .0001 *N* carbonic acid) while crappies select a concentration of from 4–6 c.c. CO_2 per liter. (3) The principal stimuli to which fishes react are H and OH ions. They do not react to ammonia, as a gas in solution.

B. Resistance Experiments.

It has been pointed out that the stock of fishes did not live well in the aquaria when these were supplied with water, which was neutral, or nearly so; to determine more exactly the reasons for the high mortality, between 50 and 60 experiments were performed. Some of these experiments lasted through a number of weeks, while others were finished in a few hours. The fishes were placed in different concentrations of acid and alkali in partly aërated water (from the aquaria) and in distilled water.

1. *Resistance to Acids.*

The resistance of fishes to carbonic acid has been worked out (Wells, '13) and it was decided to try the effects of other acids. Ten experiments with sulfuric acid in distilled water are summarized in Table III. The table shows that there is a concentra-

Table III.

Showing the Resistance of Fishes (3 Gram Blue-gills) to H_2SO_4 in Distilled Water.

Concentration of Acid.	Dying Time in Hours.
.001 *N*	3.5
.0005 *N*	7.0
.0002 *N*	42.0
.00015 *N*	60.0
.000075 *N*	Alive and vigorous at end of a month.

tion of this acid in distilled water, at which the fishes in question live as well as though in tap water. Higher concentrations of acid are fatal, the time required to kill the fishes being proportional to the hydrogen ion concentration.

2. *Resistance to Alkalies.*

In a .001 *N* solution of KOH in distilled water, a 3 gram blue-gill lived 4 hrs. and 25 min. In a .0005 *N* solution, a fish of the same size was alive at the end of 10 days. Titration at this time showed that the water had become acid to phenolphthalein from the CO_2 given off in the metabolism of the fish. The experiment was discontinued. To make sure that the fish in the first experiment had not been killed by the toxic potassium ion, another 3 gram blue-gill was placed in a .01 *N* solution of $NaHCO_3$ in distilled water. At the beginning, this solution was neutral, but it was expected that the bicarbonate would dissociate and the solution would become slightly alkaline from the carbonate thus formed. A blank control, containing the same amount of bicarbonate, but no fish, was run. The fish in the experiment died on the third day. Titration showed that the water had become .0009 *N* alkaline. The control was .001 *N* alkaline. Blue-gills therefore do not live well in water which is even very slightly alkaline.

3. *Resistance to Neutrality.*

The foregoing experiments, together with many facts recorded in the literature, suggested the possibility that the fact that it is neutral may have something to do with the toxicity of distilled water. Thirteen experiments were performed to test this possibility in a preliminary way. The facilities available did not make it possible to experiment with absolutely neutral water, but the results obtained are suggestive, as neutrality was approached very closely in some cases. Most of the experiments were performed with water that came from a copper still and will be referred to as once-distilled water. A few experiments were performed with a much purer water which was the once-distilled water redistilled in a better still and coming in contact with little copper. In neither kind of water could the amount

of copper have been especially large, however, for small blue-gills lived in both kinds as well as in tap-water, so long as the water was slightly acid.

A comparison of the conductivities of the two kinds of water showed that the once-distilled had a conductivity[1] of 600×10^{-7} while the conductivity of the twice-distilled was only 10×10^{-7}. These conductivities are for 25° C. The conductivity of the water probably does not indicate the amount of copper present however for the metal is in all likelihood present in the colloidal state. Mengarini and Scala ('12) have shown that a number of metals, including copper, form a colloidal solution with distilled water even at room temperature, and especially in the absence of air. The conditions in a still would be especially favorable for the reaction, since the temperature is high and air excluded.

The addition of an acid to a colloidal solution would tend to precipitate the colloid, and this undoubtedly explains in part the effect of addition of acid in making distilled water less toxic,[2] as it will be shown that it does. Since, however, it has been shown (Bullot, '04) that distilled water which contains no copper is still toxic to organisms, other factors must be concerned. The evidence of the experiments presented in the present paper, indicates that the neutrality of the water is one of these factors.

It has been suggested in the preceding pages that the blue-gills and crappies differ in respect to the hydrogen ion concentration which they select and their resistance to the distilled water bears out this point as the crappies die in it in a day or so, while the blue-gills live indefinitely.

(1) *Experiments with Once-distilled Water.*—This water was slightly acid to phenolphthalein and was neutral to methyl orange. Its toxicity was tested by placing fishes in jars containing a liter of the water. A 12-gram crappie died in this

[1] The conductivity of pure water is 1×10^{-7}.

[2] Locke ('95) calls attention to the fact that poisonous distilled water may lose its poisonous properties (if due to copper) by long boiling, and especially when brought into contact with sulphur, carbon, manganic oxide, cotton wool, silk, and other substances. The effect is very probably again due to the precipitation of the colloidal copper.

water in 2 days, but when this same liter of water was divided into two parts and a 3-gm. blue-gill placed in each part, both fishes were normal at the end of a month. In Table IV. is given a summary of a number of experiments performed with blue-gills in once-distilled water.

Table IV.

Showing the Resistance of Small Blue-gills (3–5 Grams) to Distilled Water that is Barely Acid with CO_2.

Conditions of Expt. Fish Placed in,	Resistance of Fishes.
1. Freshly distilled water	Normal after 5 days; expt. discont.
2. Boiled distilled water	Normal after 5 days; expt. discont.
3. Distilled water plus Na_2CO_3 to make neutral	Normal after 2 days. Water acid again.
Added a little $NaHCO_3$ to (3) to keep neutral	Dead on 10th day.
4. In dist. water as in (1)	Normal after 30 days.

Table IV. shows that the once-distilled water is not greatly if at all toxic to the blue-gills, but experiment 3 shows that these fishes cannot live in the water if it is slightly alkaline. This same distilled water is rapidly toxic to the crappies and sunfishes, however, as was shown in an experiment already described and in those which follow. This lack of resistance of the sunfishes in particular is a complete reversal of the ordinary specific resistance of these fishes as compared with the blue-gills, for in carbon monoxide, ethylene, sulphur dioxide, etc., the sunfishes are much more resistant than are the blue-gills.

On January 30, a liter of water (once-distilled) was made .00005 *N* acid with H_2SO_4 and another liter left as it came from the still. An 8-gram crappie was placed in each jar. The fish in the pure distilled water was dead in 12 hrs. while the one in the distilled water made acid, lived for 65 hrs. Several other experiments of this sort gave similar results, showing that the crappies cannot live in the neutral distilled water when it is pure, as well as they can when it is made slightly acid. It is very probable that sightly higher concentrations of acid than those used would have prolonged the lives of these fishes even more successfully than the .00005 *N* but as the stock of fishes was running low, these experiments were reserved for another time.

An experiment with small bullheads is very interesting. Normally the bullheads are perhaps the most resistant fresh-water fishes known. In the reaction experiments they selected a rather low concentration of hydrogen ion but were decidedly on the acid side of neutrality. In the pure distilled water a bullhead (4 in. long) lived 8 days; another in distilled water made .00005 *N* acid, lived for 20 days.

(2) *Resistance to Doubly Distilled Water.*—This water was less toxic to the crappies than was the once distilled water, as it contained less colloidal copper. It has been pointed out that the toxicity of the once-distilled water was lessened by the addition of acid, partly because the acid precipitated the colloidal copper. The experiments indicate further, however, that the neutrality of the water must be reckoned with also. This is again brought out, and more definitely, by a few experiments with the twice-distilled water. A quantity of this water was placed in a large bottle and a solution of barium hydroxid was suspended over it. At the end of a week the water was practically neutral. Two portions were taken in 500 c.c. Erlenmeyer flasks and a small bullhead (2.5 in.) placed in each. One portion was left neutral and the other made slightly acid with H_2SO_4. The fish in the neutral water lived 16 days and the one in the acid water 19 days. A few other experiments were performed with the twice-distilled water and all gave similar results. The stock of fishes was about exhausted, however, and further experiments were delayed until another time.

V. General Discussion.

The fact that in natural bodies of water the chemical reaction of the water may vary from alkalinity through neutrality to acidity or the reverse, makes the practical importance of a knowledge of the reactions and resistance of fishes and other organisms to such chemical conditions an obvious one. From the experiments and discussion which have gone before, it is clear that water which gives an alkaline reaction to phenolphthalein for any length of time during the year, is undesirable as a home for most fresh-water fishes. On the other hand, marine fishes (Shelford and Powers, '15) with the exception of the anadro-

mous species, probably would not survive in water which was even faintly acid. Since algæ and other phytoplankton forms (Birge and Juday, '11 and '12) may cause a body of water to become partially or wholly alkaline, through their ability to dissociate the bicarbonates, vegetation in fish waters assumes a line of importance heretofore little considered. The effects of sewage upon the acidity or alkalinity of natural bodies of water must also be reconsidered in the light of its possible injurious or beneficial effects due to its chemical reaction. Thus a large number of interesting and important questions suggest themselves.

The effect of the chemical reaction of the water upon the distribution of organisms promises much room for investigation. There is no doubt but that fishes recognize the difference between very faintly acid or very faintly alkaline, and neutral water. Henderson's work ('13), upon the mechanism which maintains a constant proportion of H and OH ions in the blood of animals, suggests the physiological reason for this extreme sensitiveness of the fishes. It is clear that even very small variations in the proportions of these two ions in the blood of the organism, are of grave importance, and we find in the blood a combination of gases and salts that makes such variations impossible as long as the animal is normal. The blood will maintain its normal chemical reaction (just on the alkaline side of neutrality) in the face of relatively large changes in the environment, yet we know that the mechanism breaks down when the change is either too great or too long continued (acclimatization is not considered at this time). The hyper-sensitiveness of the animals to the chemical reaction of the water, in the case of aquatic organisms, is another important factor in preserving the normal reaction of the blood, as the reactions of the organisms work in a way that causes them to turn back from concentrations of H or OH ion that would be detrimental. The delicacy and accuracy of these reactions are evidenced in the reaction experiments which have been discussed in the preceding pages.

The physiological effect of the acid, neutral, and alkaline water upon the organism very probably has to do with decrease or increase in the permeability of the exposed tissue cells (es-

pecially gills in case of fishes). Osterhout ('14) has shown that in plant cells alkalies increase the permeability up to death; acids however at first produce a rapid decrease in permeability, followed later by an increase which continues up to death. The concentrations of acid used by Osterhout were .001 *N* to .03 *N*. Very low concentrations such as those used in the experiments discussed here would very likely maintain a permanent decrease in the permeability of the cells, and the concentrations of acid in which the fresh-water fishes normally live, may thus protect the fishes by decreasing the permeability of their gills and preserving the normal reaction of the blood. Alkaline water, on the other hand, does not do this for fresh-water fishes, and they soon succumb in it. The results of Shelford and Powers ('15) indicate that the action of alkaline water upon marine fishes is to produce a normal permeability of the membranes and it may be that acid water would kill these fishes by decreasing the permeability below normal.

The effect of neutrality upon the permeability of tissues has not been worked out, so far as I am aware, but since fresh-water fishes, and probably marine fishes also, are negative to neutral water, it must be that such water exerts a marked effect upon the permeability, or some other physiological condition, in the gill membranes. The negativeness of organisms to neutral water indicates that they are either over-stimulated in such water, or under-stimulation sets up internal disturbances. Thus they may avoid it because of its non-stimulating character. It may well be that in neutral water, the normal chemical reactions do not go on, for acidity and alkalinity surpass all other conditions, even temperature and concentration of reacting substance, in their influence upon many chemical processes. Of all the catalytic agents, H and OH ions are by far the most important, and in their influence upon the stability of colloidal systems they are unapproached by other substances (Henderson, '13).

Birge and Juday ('11 and '12) attempt to explain the vertical distribution of the plankton in the lakes of Wisconsin and New York, upon the basis of relation to oxygen and food. This attempt has, it seems to me, met with little success, and they

themselves point out many contradictions. According to their idea, the plankton forms must in many instances be reacting positively to concentrations of oxygen which are as small as .1 c.c. per liter, or even less. This supposition is contrary to all the experimental evidence regarding the reactions of aërobic fresh-water organisms to this gas. In an attempt to correlate the distribution of the zoöplankton with the chemical reaction of the water, I have gone over Birge and Juday's tables and figures, and have come to the conclusion that such correlation exists. Their data indicate in practically all of the lakes (in the summer condition) a point at some depth below the surface of the lake, where the organisms are more numerous than at any other depth. In many cases this rise is proportionately very high and is usually of small amplitude. Thus the large number of forms occurs in a rather limited region vertically. After the rise, there is a marked diminution in the number of forms and then again at a little greater depth there is another increase, smaller than the first, but still very noticeable in their curves. This increase is followed by a second diminution. The first diminution usually occurs in or near the thermocline where the temperature often shows a very sudden lowering. The oxygen supply sometimes falls off here also, but not always, and in the lakes to which I refer particularly, the oxygen supply is practically the same at all depths. A very important fact, however, is that the water in the region of the thermocline, *i. e.*, at the region of smallest numbers of plankton, is often neutral or very nearly so (summer condition). Above this region the water is alkaline, and below acid. From the data given in Birge and Juday's Tables XVIII. and XIX. ('12, pp. 602–608), I have compiled the following table (Table IV.) to show the relation of the zoöplankton to this neutral region. Birge and Juday's Table XVIII. is a record of temperatures and gas contents at the different depths; Table XIX. is an analysis of the plankton catches made in ten lakes. The records for a given lake were all made on the same day. Table XVIII. gives titration records which show that in three of the lakes at a definite depth, the water was neutral. Table XIX. gives the plankton collections at different depths in these three lakes, on the same

day. Table IV. inserted below, is made up from a combination of the data found in the two tables; most of the data in Table IV. refer to the three lakes in question. In the instance of *Triarthra*, however, the data come from two other lakes, as this form does not occur in the three lakes from which the other data are taken.

Table IV.

Showing the Relation of Zoöplankton to Neutrality.

The table is compiled from Birge and Juday's ('12) Tables XVIII and XIX, Most of the data are taken from their records for the three lakes, Canandaigua, Seneca, and Skaneateles. In these lakes the neutral depth was accurately located by titration of samples. The titrations and plankton collections were made on the same say. The data for the rotifer triarthra are taken from lakes Hemlock and Keuka as these are the only lakes in which this form occurs in the records. n.c. = no collection at this degree of acidity or alkalinity. The figures in the columns indicate the number of forms per cubic meter of water.

Name of Animal.	Alkalinity in C.c. per Liter of CO_2 to Make Neutral.			Neutrality.	Acidity in C.c. of CO_2 per Liter.		
	3-2.	1.5-1.	.5-.25.	0.	.25-5.	.75-1.	.1-1.5.
Pleosoma (R)	3,925	0	0	0	0	0	0
Vorticella (P)	12,250	0	0	0	0	0	0
Asplanchna (R)	11,320	400	0	0	0	0	0
Dinobryon (P)	43,700	19,130	42,800	0	0	0	0
Diaphanosoma (C)	2,885	2,750	n.c.	260	0	0	0
Nauplei	28,150	28,050	13,250	570	140	40	205
Diaptomus (Co)	7,850	6,660	17,350	2,220	1,440	390	100
Conochilus (R)	130	290	250	250	30	65	30
Anuroea (P)	4,000	1,250	200	30	20	20	20
Cyclops (Co)	13,775	7,620	7,620	25	30	0	5
Notholca (R)	625	685	65	0	65	0	0
Daphnia (C)	1,260	650	400	130	1,145	25	0
Ceratium (P)	52,330	104,500	85,160	2,025	11,760	5,750	1,670
Polyarthra (R)	12,350	1,620	2,350	160	1,190	1,240	40
Malamonas (P)	0	n.c.	770,400	95,600	1,900	0	1,900
Triarthra (R)	0	n.c.	0	n.c.	1,050	1,110	2,425

Letters in parenthesis after name of animal indicate the following. (R) = Rotifer; (P) = Protozoan; (C) = Cladoceran; (Co) = Copepod.

Table IV. shows (1) that all the zoöplankton forms are more numerous on either the acid or the alkaline side of neutrality, than they are at neutrality itself, *i. e., they are negative to neutrality;* (2) some forms as *Pleosoma* and *Vorticella*, are found only in the alkaline water; (3) others range between slight alkalinity and slight acidity but are never very numerous at neutrality and often (*Daphnia*, *Ceratium*, etc.) show an increase on either side; (4) a few forms (*Triarthra*) occur wholly on the acid side of neutrality.

The factors that regulate the distribution of the plankton in the lakes are undoubtedly numerous. The only certain way to determine them is to investigate experimentally the reactions of the animals to the factors concerned, both singly and in combination. To do this would be tedious but not especially difficult. As an index to the distribution of these forms, I believe that the presence and position of a neutral layer of water will be found to be important.

Besides the experimental data presented in the papers by Birge and Juday, the literature contains much other experimental evidence which bears directly upon the question of the toxicity of neutrality to organisms. Much of this evidence is found in connection with experiments upon the toxic effects of distilled water, and the action of salts in antagonizing this toxicity. In a series of papers published by Ringer and his students between the years 1883 and 1893 the question of the toxicity of distilled water was investigated and its reality apparently demonstrated. It was also shown that various salts are effective in neutralizing this toxicity, some being much more efficacious than others. In 1893 Naegeli showed that for Algæ (*Spirogyra*) at least, the toxicity of distilled water was due to contamination from the copper stills in which it was prepared. Locke ('95) confirmed Naegeli's results by showing the effect upon certain fresh-water animals to be due also to the minute amounts of copper present, and Ringer ('97) again taking up the subject reversed his former conclusions and confirmed those of Locke. Jennings ('97) found that *Paramœcia* live for weeks in distilled water. Moore ('00) says that young trout and tadpoles (unfed) live as long in distilled as in tap water, *i. e.*, several weeks. Lillie ('00) says that *Planaria maculata* will live in distilled water. Pure distilled water seemed then not to be toxic to fresh-water animals though apparently toxic to most marine animals. *Fundulus* eggs seem to be an exception among marine animals (Loeb, '99), as they can live in distilled water for weeks and still produce normal embryos. In 1903 Bullot after testing the effects of distilled water upon the fresh-water amphipod, *Gammarus* concluded that pure distilled water was toxic to this crustacean. Bullot's experiments were performed with great

care; he considered and seemed to have eliminated the following possible toxic factors: copper, impurities from the glass, low oxygen, ammonia, and carbon dioxide. He found also that NaCl in small concentrations would neutralize the toxicity of the pure water to such an extent that the animals lived almost as well in an .00008 *N* solution of this salt as in the natural fresh water. The toxicity of pure distilled water, he concluded, is due to the lack of salts in solution. Peters in 1908 performed some very careful experiments to test the effect of pure distilled water upon protozoa. He came to the conclusion that distilled water which contains no salts, and which is changed often enough to prevent their accumulation from the metabolism of the animals, is rapidly toxic to these forms.

I have gone over the above papers and have found many statements which indicate that the presence of a certain concentration of hydrogen ion, was beneficial to the animals experimented upon. For instance, Ringer ('83) states that the distilled water which he used killed minnows, on an average, in 4.5 hours. He also says that the distilled water was very faintly acid; so faint was the acidity that he did not rely upon his own judgment but had others make the test also. However, he says, to prove that the acidity was not the cause of the death of the minnows, he took three liters of water and to one added 6 drops of 10 per cent. acetic acid, to the next 12 drops and to the third 20 drops. He then placed three minnows in each liter of acid solution. After 24 hours, the minnows were "quite natural" and he concluded, therefore, that the acidity could not have been harmful in the case of the distilled water. This conclusion of Ringer's illustrates the attitude taken by most authors with regard to the presence of acid in the water, that is, the acid is looked upon as a detrimental factor, to be considered negatively. So far as I have been able to read, the authors quoted above have taken little consideration of the possibility that the presence of a certain concentration of H or OH ion is essential to the welfare of animals.

This Ringer does not suggest, even though the minnows in the acid water were well on the way to live as long as any of the animals kept in salt solutions. In this same paper, Ringer notes

that when he put a large number of fishes (up to a maximum) into a given volume of distilled water, they lived longer than one or two fishes placed in the same volume of water. He attributes this to the excretion by the fishes of inorganic salts, and does not take into consideration the carbon dioxide factor which would have increased the acidity of the water to many times that of the almost neutral distilled water. Again, in speaking of the salts which are best for preserving life in distilled water, Ringer states that the calcium salts are better than those of sodium and potassium, that $CaSo_4$ is better than $CaCl_2$ and that the phosphate of lime $(Ca_3PO_4)_2$ is much superior to all the other salts. This latter salt, he states, is decidedly acid, and he says ('86) "it is interesting to observe that though the circulating fluid with phosphate of lime gives a slight acid reaction to delicate blue litmus paper it will sustain contractility of muscle for hours." Thus a small hydrogen ion concentration seems to be beneficial, if not essential, to the continued life and activity of the organisms and tissues in question.

The question of the existence of a carbon dioxide optimum for animals has received considerable investigation with varying results. Ringer in 1893 investigated the influence of carbonic acid upon the frog's heart and concluded that free CO_2 in saline solution arrests cardiac contractility. He does not state what concentrations of CO_2 were used, but since he speaks of passing carbonic acid through the solution "for some time," his solutions were probably very acid. In a few experiments he neutralized the slightly acid distilled water which was used to make up the saline solutions, with NaOH, and noted that in this neutral solution, the contractions of the heart very soon became abnormal. Jerusalem and Starling ('10) review the literature regarding the importance of carbon dioxide for the ordinary functions of the body, and report a series of experiments to determine its influence upon the beat of the heart of the frog, tortoise and mammal (cat). They conclude that the CO_2 tension in the blood must be maintained at a certain height, if the pumping action of the heart is to be normally carried out. In their review of the literature they point out that their conclusions are in accord with those of Miescher, Haldane, Mosso, Hender-

son, and Bottazzi (see pp. 279–280). The lowest concentration which Jerusalem and Starling used was 2 per cent. of an atmosphere or about 20 c.c. CO_2 per liter. Their highest ran up to 200 c.c. per liter. Hooker ('12) tested the effect of carbon dioxide upon muscular tone and, in opposition to Jerusalem and Starling, concluded that this gas does not appear to be directly beneficial to tissues, except in case of intestinal muscle rhythm. He thinks it may be indirectly beneficial. Like most other workers upon this problem, Hooker used very high concentrations of the gas. His concentrations varied from 5 per cent. to 20 per cent. of the gas, in the atmosphere to which the solution bathing the tissue was exposed. Water will dissolve nearly its own volume of CO_2 and thus the concentration of carbonic acid varied from 50 to 200 c.c. of CO_2 per liter. The smallest concentration used would kill most fresh-water fishes in a short time.

Reuss ('10) worked upon the effect of CO_2 upon the respiration of fishes and concluded that it is an important one. The regulation according to him is through the respiratory center and not peripheral as Bethe ('03) believed. Shelford and Allee ('13*a*) note the extreme sensitiveness of fishes to CO_2 in gradients, and think the production of the gas as a product of the metabolism of the organism may tend to increase its external effect when the fishes come in contact with water containing it.

Bullot ('04) in his work with the fresh-water amphipod (*Gammarus*) noted, as did Ringer in the case of fishes, that the animals lived longer in distilled water when a number was present in a given volume, or in other words, when the volume of water per individual was small. He says: "If the amount of water falls below a certain limit, the animals will live the longer, the smaller the amount of water, provided the quantity does not fall below a certain minimum." In Table V. I have collected Bullot's data showing this point. The table shows that the relation holds for *both redistilled water and water distilled in copper alone*. The length of life in the water from the copper still is proportionately shorter throughout.

Table V.

Showing the Resistance of Amphipods in Distilled Water, when Equal Numbers of Animals are Placed in Different Volumes of Water, or when Different Numbers of Animals are Placed in Equal Volumes of Water (Compiled from Bullot, '04, pp. 204–5).

Per Cent. of Animals Alive After 2 Days.

No. Animals.	Volume of Water.	Water. Redistilled in Glass.	Water. Distilled in Copper.
1	Same throughout	45%	Proportionately less throughout.
5		80%	
10		90%	

Time Required to Kill One Half the Animals.

No. Animals.	Volume of Water.	Redistilled in Glass.	Distilled in Copper.
Same throughout	5 c.c.	10 days	8 days
	20 c.c.	2.5 days	1.5 days
	50 c.c.	1.5 days	1 day
	100 c.c.	same	same.

In considering the possible importance of CO_2 as a factor in the toxicity of distilled water, Bullot states that the water which he used was very faintly acid to phenolphthalein, but not enough to injure the animals. He says: "We know from the works of P. Bert that, for cold-blooded animals like the frog, for instance, CO_2 is toxic only when its concentration in the air reaches 15 per cent. This corresponds to a solution of 15 per cent. of this gas by volume, as the water dissolves its own volume of CO_2 at ordinary temperature and normal pressure. This quantity is 350 times larger than the one which could be found in the distilled water." A 15 per cent. solution of CO_2 means 150 c.c. per liter of water. 1/350 of this is .42 c.c. In other words the distilled water used by Bullot was practically neutral, since the amount of hydrogen ion to be obtained from so small a quantity of so little ionized an acid as carbonic acid, would be almost negligible. In the gradient experiments cited in this paper, I have shown that certain fishes are negative to so small a concentration of CO_2 as 1 c.c. per liter, in preference for slightly higher concentrations. I have further shown that these fishes do not live as well in distilled water that is practically neutral, as they do in the same water made slightly (.00005 *N*) acid. Thus the existence of a hydrogen ion concentration optimum for these forms seems to be clearly demonstrated.

Peters ('08) makes no mention of the possibility of the neutrality of the distilled water which he used, having something to do with its toxicity, yet in a previous paper ('07) he recognizes the importance of the presence of a certain concentration of hydrogen ion for the existence of certain protozoa in hay infusions. On page 346, he says: "The data obtained indicate that, of the chemical conditions, the concentration of acid . . . is one of the chief factors determining the biological content and history of a culture."

From the data and discussion that have gone before, it seems certain that the chemical reaction of the water is a factor of marked importance in the life history of fresh-water animals. Some fresh-water forms are apparently positive to alkalinity as seen in the fresh-water lakes (Birge and Juday, *loc. cit.*) and others, that normally live in water that is acid with CO_2 are not killed by living in alkaline water (isopods). On the other hand, many forms, and probably most of the fresh-water fishes belong here, are always found in acid water if such be available, and these forms cannot live normally in neutral to alkaline water. Shelford and Powers ('15) have shown that marine fishes select the alkaline side of neutrality in a gradient, and in this difference in the behavior of the fishes, lies a key to the fundamental physiological difference in the organisms of these two habitats. Fresh-water fishes must live in the presence of an excess of hydrogen ion if their life processes are to be carried on in normal fashion. Shelford ('14) states that the carbon dioxide content of the water over the breeding grounds of fresh-water fishes should not average more than 1 c.c. per liter, nor exceed 5 c.c. during the summer months. This statement is probably wrong in limiting the average to 1 c.c. per liter for some fishes, as the green spotted sunfish and the crappies are negative to this small concentration of CO_2 showing a preference for slightly higher concentrations. Blue-gills, on the other hand, select a degree of acidity that is very little above neutrality. The CO_2 concentration selected by fishes probably varies with the season, and certainly with the salt content of the water in which they live. The variations of the CO_2 optimum in salt concentration will be discussed in the second paper of the series.

One thing is clear; the chemical reaction of the water should be known with accuracy, in all experiments with salts and gases in solution. A recognition of this fact will help to clear up some of the many contradictory results which have been obtained by various workers. It seems to be demonstrated beyond a doubt that the toxicity of distilled water is in part due to the absence or scarcity of inorganic salts, but it is also evident that the neutrality of such water may be an important factor in its toxicity.

VI. GENERAL CONCLUSIONS.

1. The chemical reaction of the water is an important factor in the reactions and resistance of organisms.

2. Fresh-water fishes select slight acidity in a gradient, when the other choices are neutrality and alkalinity. They choose slight alkalinity in preference to neutrality.

3. The CO_2 optimum for the different species of fishes experimented upon, varies from very close to neutrality for the blue-gill, to 6 c.c. per liter for the sunfishes and crappies. This is for the November to January condition. At other seasons and in other waters, the optimum probably varies somewhat. The optimum acid concentration for fresh-water fishes in distilled water is about .00005 N H_2SO_4.

4. The distribution of the plankton in the lakes of Wisconsin and New York (Birge and Juday, '11 and '12) shows a very interesting correlation with the chemical reaction of the water. There are fewer animals at neutrality than in the slightly alkaline and slightly acid water just above and below the neutral layer. Thus the forms are negative to neutrality.

5. The neutrality of distilled water is a factor to be considered in its toxicity.

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